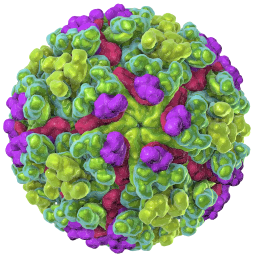
UNIT: CHIKUNGUNYA

LESSON 1: SILLY NAME, SERIOUS VIRUS

Activity 1A: Of Mice or Men… and More

TEXAS BIOMEDICAL RESEARCH INSTITUTE
HEALTH STARTS WITH SCIENCE



CHIKV Virus

The ***chikungunya*** virus (chic-un-GOON-yah; CHIKV) is a virus that is carried by mosquitos. Specifically, CHIKV is carried by the Aedes mosquito. This mosquito is a vector for many different viral diseases. A vector is an organism that carries a virus and infects another type of organism. The Ades mosquito is also a vector for dengue fever and Zika virus.

The name chikungunya is derived from the Kimakonde language, which is spoken by people living in southern Tanzania, a country located on the continent of Africa. In Kimakonde, the word chikungunya translates "to become contorted". This makes sense when considering some of the symptoms experienced when infected by CHIKV result in joint pain, specifically in the hands, feet, and knees. The joints become inflamed causing excruciating pain which causes the individual to "contort" their body in response to the pain.

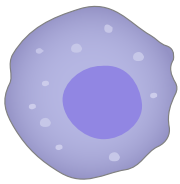
For most people infected by CHIKV, it takes up to 12 days after being bitten by the mosquito before symptoms occur. This time is the incubation period: the amount of time it takes for the virus to replicate before symptoms are seen. The symptoms include fever, headache, muscle pain, rash, nausea, vomiting, and joint pain. For many people the symptoms come on quickly and don't last very long. This is an acute case of CHIKV. But for some people, the symptoms can last much longer and is called chronic. The joint pain can progress into something more serious: rheumatoid arthritis. The condition causes stiffness and pain, lasting for months to years.

The structure of CHIKV makes it an alphavirus. Alphaviruses contain single strands of RNA. The single-stranded RNA is contained inside a lipid membrane. Unlike other types of viruses with a keratin capsule, the outside of the CHIKV is not hard. It is made of lipids which is a type of fat. The charges on the atoms that make up the lipids have electrical charges. The charges are not equally distributed between the atoms in the molecule, creating polar ***covalent bonds***. In a polar covalent bond, electrons are not shared equally with some atoms having a stronger pull or ***affinity*** for electrons than others. The polar bonds between molecules attract keep the icosahedral shape even as the

When the mosquito pierces the skin, it injects an anticoagulant, to keep your blood from clotting. This allows the blood to flow from under the skin into the mosquito. The CHIKV is carried in the anticoagulant and enters the circulatory system. Once in the blood, the CHIKV finds its way into cells through a process called receptor-mediated endocytosis. During this process, a glycoprotein on the membrane of CHIKV binds to a specific receptor on a cell. The glycoprotein on CHIKV looks a lot like a glycoprotein found in our bodies which "tricks" the cell and causes it to bind to the virus. The virus is then absorbed into the cell. The pH level inside the cell triggers viral replication. As the virus replicates, each separate viral RNA

is enclosed in membranes produced by the cell. These are called "endosomes". The endosome then fuses with the outer membrane of the cell and releases the viruses. Each released virus can repeat the process as it infects other cells.

Not every organism responds in the same way to viral infection due to differences in how immune systems respond. In this study scientists compared the response of humans to mice when infected with CHIKV. One way to assess differences in immune responses of different organisms is to use ***in vitro*** techniques. In vitro techniques are scientific processes performed in a controlled environment, conducted in petri dishes or test tubes, rather than a living organism! Tissue for in vitro studies are obtained from living organisms which are cultured (grown outside the body). Samples of tissue sources may be obtained through blood draws or skin scrapings.



Macrophage

For this study, scientists obtained immune cells from mice and cells from humans and placed the samples in separate petri dishes. The cultured immune cells, known as ***macrophages***, from both species were infected with CHIKV. Much of the scientific process centers on observations, interpreting what these observations mean, and what additional questions are generated. Following the scientific process, scientists observed the in vitro samples for responses to the CHIKV infection. They compared the rate at which the macrophages were infected with CHIKV and observed differences in responses, referred to as the ***mode of infection*** (MOI).

In both samples, scientists initially observed a drop in the amount of CHIKV virus in the culture dishes. This is due to the CHIKV virus entering and replicating in the cells. This was observed in both murine and human macrophages. However, over a few hours, the amount of CHIKV present increased for both organisms. This indicates the virus replicated inside the macrophages, causing the macrophage to burst which released more CHIKV into the culture dish. Over the course of two days, 48 hours, scientists observed different MOIs for the samples. Human macrophage samples had a higher MOI than the murine samples. From this observation, scientists interpreted this to mean that CHIKV replicates more efficiently in human macrophages than in murine macrophages.

But scientists do not rely on only one test. They took a closer look at the few hours where the CHIKV initially entered the cells of both samples. They evaluated the amount of CHIKV RNA present during the four-hour replication period: 2 hours to 6 hours post infection. At the 8-hour mark post infection, scientists used quantitatively tested the amount of CHIKV RNA present in murine macrophages and human macrophages. There was a significant difference in MOI levels. The amount of CHIKV RNA present in human macrophages was exponentially higher than that found in murine macrophages.

Macrophages are the first line of defense against infection. Observing an exponential difference between murine and human macrophage response led scientists to conclude that the human immune response to CHIKV is much different than that of mice. All immune systems contain interleukins (IL). These specialized cells are messengers that send signals which instruct the immune system how to respond to an invader, like CHIKV. Further testing of the in vitro samples provided quantitative data indicating all human ILs signal the immune

system that cause an inflammatory response. However, a majority of the murine ILs sent signals caused an anti-inflammatory response.

Based on the data from these different tests, scientists concluded murine immune response to CHIKV infection is different than that of human immune systems. This study provides evidence that the pro-inflammatory response of the human immune system may be the catalyst that triggers persistent joint pain and arthritis from CHIKV infection.